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Radiatsionnyi diploidnyi ginogenez u gorbushi

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Radiation-induced diploid gynogenesis in the pink salmon

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After the mechanism of chromosomal sex determination became known (Bridges, 1925), the idea arose to separate spermatozoa bearing the X- and Y-chromosomes by some kind of appropriate method (Kol'tsov, 1936). This problem has not yet been resolved.

Considerable success has been achieved with experimental sex change in mammals and birds by means of the sex hormones. However, sex inversion obtained by the transplantation of gonads to individuals of the opposite sex was only of brief duration because of the rejection of the foreign tissue by the recipient organism. The injection of sex hormones also did not resolve the problem, since the effect of these hormones terminated when they ceased to be administered. The principal deficiency of these and similar attempts was that the genetic constitution of the experimental animals did not change, which means that there was no true genetic sex change.

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* The numbers in the right-hand margin indicate the corresponding pages in the original text. (Tr.).

It has been possible to achieve much greater success with experimental control of sex in the silkworm (Astaurov, 1968). By means of artificial parthenogenesis and experimental androgenesis it has been possible to obtain, under laboratory conditions, either only females or only males of the silkworm. Subsequently, following studies using the method of chromosomal translocations, success was achieved on a commercial scale in the production of silkworm males, which have a greater productivity of raw-silk than the females (Strunnikov, 1971).

The Japanese geneticist Yamamoto (Yamamoto, 1953; Yamamoto and Kajishima, 1968) was able not only to obtain a stable phenotypic sex change in the medaka (Orizias latipes) and goldfish (Carassius auratus) after feeding the fry of these fish with food containing male and female sex hormones, but also to obtain progeny, consisting 100% of genetic females, from revertant males with an XX genetic structure. 152

The pink salmon, one of the species of Pacific salmon of the genus Oncorhynchus, is differentiated from other species of this genus by certain unique features of its development. It has been established that all of the fry of the pink salmon, up to a certain period of their development, are females on the basis of the structure of the gonads. Then in some of the fry (obviously, in the genetic males) the ovaries degenerate and subsequently the gonad develops according to the testis type (Persov, 1965). Thus, it is impossible to determine the sex in the fry of the pink salmon up to the time of the redifferentiation of the gonads, since there is no sexual dimorphism in the sexually immature pink salmon. This difficulty may be overcome if one artificially obtains a race with some feature

that is associated with the sex. Moreover, it is not known as to which type of genetic sex determination occurs in the pink salmon (and also in the other Pacific salmon), whether it is of the XY or WZ type.

Let us consider the possible ways of obtaining females artificially. Since it is impossible to differentiate the fry of the pink salmon according to their sex in the early stages of development, the first stage is to resolve the problem of obtaining experimental material that is homogeneous as to the sex.

Artificial gynogenesis. The egg cells (roe) of a sexually mature pink salmon are fertilized with previously irradiated (to inactivate the nuclear material) spermatozoa of pink salmon males. The spermatozoa, penetrating into the egg cells, stimulate the latter to parthenogenetic development, as a consequence of which two types of result are possible.

1. Female individuals have an XX chromosomal formula. In this case all of the developing individuals will be of the female sex:

$$♀\ XX \rightarrow X \rightarrow ♀♀\ XX,$$

2. Female individuals have a WZ chromosomal formula. In this case the egg cell bears either a Z chromosome (ova "for males") or a W chromosome (ova "for females"). Two variants are therefore possible:

a) ♀ WZ — Z — ♂♂ ZZ;

b) ♀ WZ — W — WW - a zygote that does not exist in nature and which is not viable.

Thus, in this case all of the developing individuals will be males. This experiment permits a provisional answer

to the question concerning the type of genetic sex determination in the pink salmon. The principal difficulty in realizing this consists of obtaining gynogenetic embryos that have a diploid set of chromosomes (haploid embryos, as a rule, are not viable).

Hormonal sex change. If sex in the pink salmon is determined according to the XY type, the obtained individuals of the female sex are treated in the fry stage with male sex hormone (testosterone or methyl testosterone) in order to change the sex. The obtained revertant males will be full-fledged phenotypic males, though their genetic structure remains unchanged, i.e. of the female type (XX). If such a revertant male is crossed with a wild female of the pink salmon, only females should appear in the progeny:

XX revertant male x X'X' wild female = ♀♀ XX'.

All of the individuals, as is evident from the schema, are true, i.e. genetic diploid females, and this effect is achieved in the second generation. If sex in the pink salmon is determined according to the WZ type, then the crossing of a revertant male with a wild female should produce the following result:

WZ revertant male x W'Z' wild female = ♂ZZ' + ♀W'Z + ♀WZ + WW'.

The sex ratio in this case will be 2:1 in favour of the females, though a quarter of the zygotes (WW') should die.

Blocking redifferentiation of the gonads in the pink salmon. In connection with the fact that chromosomal (genetic) sex determination in fishes is not as "rigid" as, for example, in mammals, sex changes are possible in fish developing in different ecological conditions. Cases are known of spontaneous redifferentiation of the gonads in certain fishes (Kirpichnikov,

1974). For example, females of the Bauntov whitefish* were transformed into phenotypic males under the influence of a lowered temperature and a deficiency of feeding (Anpilova, 1965). Therefore it is not without sense to attempt to block the redifferentiation of the gonads in the pink salmon by changing the temperature conditions for holding its alevins and fry during the period preceding such redifferentiation. In this case the effect would be achieved already in the first generation. However, even if this were to succeed, half of the obtained females would be genetic males, and on crossing 154
them with wild males the following variants are possible:

1) XY-type of sex determination:

XY revertant female x X'Y' wild male = ♀ XX' + ♂ XY' + ♂ X'Y + ♂ YY'.

YY' is an exceptional zygote which, if it survives, may develop along the male type. The sex ratio in this case will be 3:1 in favour of the males. Since the remaining 50% of individuals are genetic females, which will give a sex ratio of 1:1 in their progeny, the total number of females in the progeny would be raised to 37%.

2) WZ-type of sex determination:

ZZ revertant female x Z'Z' wild male = ♂♂ Z'Z + ♂♂ ZZ',

i.e. only males. Therefore it is scarcely desirable to block the redifferentiation of the gonads in pink salmon, since this may lead to increasing the number of males in natural populations. Theoretically, however, such an experiment is of interest.

In conducting laboratory experiments according to the schemes described above, the the task of early diagnosis of the sex acquires considerable importance. We will list only the

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* Coregonus lavaretus baunti. (Tr.).

principal possible leads for solving this task:

1) staining the sex chromosomes with specific stains;

2) the immunological reaction with Y-antigen (non-species-specific antigen to the Y-chromosome);

3) identification of Barr bodies (sex chromatin);

4) cytophotometric differentiation of spermatozoa bearing different sex chromosomes.

In addition, it is necessary to have a morphological control (including features at the ultrastructural level) of the development of the embryos, alevins and fry. An independent problem (though providing difficulties only of a technical character) is the maintenance of the experimental individuals in aquaria up to the sexually mature state.

The above-described variant of radiation-induced diploid gynogenesis was tested by us in 1977 in the Sokolovskii fish farm of the Sakhalin Piscicultural Enterprise and produced positive results. For the inactivation of the genetic material in the spermatozoa the milt of the pink salmon was irradiated in a "Stebel'-ZA" γ-installation at dosages of from 10 to 150 krad. The viability of the irradiated spermatozoa was determined under the microscope on the basis of the retention of motility after activation by water. 155

To obtain the gynogenetic progeny, the eggs of sexually mature pink salmon females were fertilized with the irradiated spermatozoa. Since, as a rule, non-viable haploid embryos develop following fertilization by spermatozoa whose genetic material has been inactivated by radiation, we employed temperature shocks which permitted us to considerably increase the number of viable diploid embryos. The regimes and durations

for carrying out the temperature shocks were determined by a series of experiments.

The cytogenetic analysis of the developing embryos and fry, carried out in the winter and spring of 1978, showed that both haploid and diploid specimens were present among the experimental individuals. After the necessary tests and improvements have been made, the described method may, in our opinion, be recommended for obtaining pink salmon females on a large scale under commercial conditions.

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